Chapter 15
Euprymna hyllebergi and *Euprymna tasmanica*

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Abstract Bobtail squids of the genus *Euprymna* are small in size with a benthic habit. Such small size results in their insignificance in fisheries and aquaculture focused for human consumption. The unique ability of the voluntary adhesion system and symbiotic bacteria used for bioluminescence is now a primary research focus with potential industrial and biomedical applications. Their small size is well suited for the home aquarium with small volume. Culture of this cephalopod group can therefore serve both research and recreational purposes. Aquaculture in the laboratory provides valuable information for culture methodology that is utilized throughout the entire life cycle of several consecutive generations. This small size and benthic habit of *Euprymna* are advantageous for small-scale closed or open seawater culture systems. Major trends for culturing *Euprymna* are similar to other cephalopod groups, particularly benthic octopus that also produce planktonic hatchlings. Reduction of the cost of production is necessary for future large-scale production, with novel protocols for live feed requirements of planktonic young in the nursing phase.

Keywords *Euprymna* · Small size · Benthic habit · Small-scale culture · Closed and open seawater systems · Research and recreational purposes

15.1 Importance of the Species

The Thai bobtail squid, *Euprymna hyllebergi* Nateewathana 1997, is a common species occurring in the Andaman Sea of Thailand (Indian Ocean) and the Gulf of Thailand (Pacific Ocean; Nateewathana 1997; Nateewathana et al. 2001; Aungtonya et al. 2011). This species is small (20–40-mm mantle length, ML),

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Fig. 15.1 Partial sand-coated *Euprymna hyllebergi* shortly after emerging from its burrowing site. (Photograph of J Nabhitabhata)

neritic and strictly nektobenthic, inhabiting coastal waters in a similar manner to its congeners that occur in the Indo-west Pacific region (Summers 1985; Norman and Lu 1997; Reid and Jereb 2005). *E. tasmanica* (Pfeffer 1884), otherwise known as the "southern dumpling squid", is a species that resides around the continent of Australia and is found in similar habitats as *E. hyllebergi* (Reid and Jereb 2005). *E. tasmanica* is somewhat larger in size than *E. hyllebergi,* with an approximate ML of 30–40 mm (Norman and Lu 1997). In Thailand, *E. hyllebergi* and congeners are captured as by-catch of commercial fishing, particularly push netting and trawling (Nateewathana 1997). The yields are discarded as trash fishes due to their small size and low economic value. Because of this, fishing statistics of both species are not available (Nateewathana et al. 2001; Reid and Norman 1998; Reid and Jereb 2005).

Although the economic value of *Euprymna* as human food is low, there is a growing importance of the animals as scientific experimental models. The unique behaviour of *Euprymna* is its capability to retain a "coat" of sand or other debris on its dorsum (Fig. 15.1) when it emerges from its daily buried state to hunt prey at night (Anderson et al. 2002). The function of the sand coat is presumably for camouflage, making the squid difficult to be detected visually by its predators and prey (Anderson and Mather 1996; Shears 1988). The stickiness of the sand coat depends upon secretions of the ectodermal epithelium (Moynihan 1982). Choice between being sticky and nonsticky is voluntary and variable (Moynihan 1982). This indicates that the ability to use a sand coat might have evolved from the initial use of the behaviour for sand consolidation when the squid buries (Shears 1988). The ability to begin sand coating starts 5–7 days after hatching, simultaneous to their burrowing ability (Nabhitabhata et al. 2005). Additionally, bobtail squids are presently used as a model organism to identify a new generation of biomimetic adhesives and marine antifouling compounds with potential industrial value (Byern and Grunwald 2010).

Symbiotic associations between *Euprymna* and the bioluminescent bacterium *Vibrio fischeri* has been a recent focus as a model for investigating the process of bacterial colonization of host tissues and its effect on host development (Ruby 1999, Ruby and Lee 1998). *V. fischeri* and other luminous bacteria form a variety of pathogenic and cooperative associations with marine animals; more recently, they are being increasingly recognized as causes of invertebrate diseases (Guerrero-Ferreira

and Nishiguchi 2011; Guerrero-Ferreira et al. 2012). Since the process of bacterial colonization of the squid light organ begins immediately after hatching (Ruby and McFall-Ngai 1992), independent aquaculture of the squids and their luminous bacterial partners could yield valuable results for biotechnological and biomedical sciences (McFall-Ngai et al. 2012; Nyholm and Nishiguchi 2008). Because of these newly developed models for basic research, the advancement of culturing techniques for species of *Euprymna* has been especially important for monitoring fitness between generations, effects of inbreeding and, more importantly, diet and stress under laboratory conditions (Nabhitabhata et al. 2005; Sinn 2005; Sinn et al. 2008; Moltschaniwskyj and Carter 2010). Additionally, more information on their development, growth and time to reproduction can indicate whether all species have similar life-history strategies, and if this is dependent upon habitat or other abiotic factors.

15.2 State of the Art

Both *E. hyllebergi* and *E. tasmanica* are small in size and found living within benthic habitats. This is an advantage to provide culture conditions on a smaller scale with lower cost and less requirement of facilities compared to those used for pelagic and large-sized species. *Euprymna* can be cultured through several consecutive generations ensuring a supply of broodstocks. Broodstocks collected from the wild can spawn in captivity and are maintained throughout the life history of the animal. *E. hyllebergi* hatchlings are fed with wild-collected live feed for approximately 30 days, but later can be trained to accept dead feed. *E. tasmanica* hatchlings are fed small mysid shrimp two times a day for approximately 6 weeks, and then moved to a diet of ghost shrimp for the duration of their lives while in captivity. Interestingly, *E. tasmanica* adults were not trained to feed on dead material, and prefer not to eat food items that do not move. These same facilities for raising juvenile squids can be used for culture throughout the squid's entire life cycle. The daily growth rate for *E. hyllebergi* is 2.4% in length and 7.5% in weight through the culture period of 100 days. Growth rates for *E. tasmanica* were approximately 3.5% in length and 10% in weight for approximately 60 days. Growing demands for these squid for use as both biotechnological and biomimetic experimental models as well as ornamental animals for home aquaria and teaching laboratories are beneficial for aquaculture and biomedicine, e.g. Nabhitabhata et al. (2005), Moltschaniwskyj et al. (2007), Sinn and Moltschaniwskyj (2005) and Sinn et al. (2008).

15.3 Broodstocks Maintenance

Broodstocks of the Thai bobtail squid, *E. hyllebergi,* are collected live from otter board trawlers and beam trawlers, operating along the eastern part of the Gulf of Thailand, South China Sea and Pacific Ocean. Onboard, the squids are maintained in cylindrical fibreglass tanks of 50-L capacity containing 30 L of fresh seawater

with aeration and then, upon landing, are transported to the cephalopod hatchery. The broodstocks are maintained in an open system of cylindrical concrete tanks of 2 m^3 with flow-through filtered seawater (for the seawater supply system in this chapter, see also Chap. 7 "Aquaculture to Restocking"). Artificial substrates, made from pieces of longitudinal-cut polyvinyl chloride (PVC) pipe (50-mm diameter, 150-mm length), are previously placed on the tank bottom as shelters or "dens".

Broodstocks of southern dumpling squids, *E. tasmanica,* are collected by seine net in shallow waters of Botany Bay, New South Wales, Australia. Adult animals are transported to running open seawater facilities located at the Sydney Institute of Marine Sciences (SIMS) at Chowder Bay, NSW. Adults are acclimated to the conditions in the tanks (34 psu, 18 °C) and transported to New Mexico State University within 2–3 days. Transport of the squids takes approximately 36 h tank to tank in aquaria bags with less than 1 L of seawater. Animals are then acclimated to the contained recirculating artificial seawater tanks (100 L) at New Mexico State University under the same culturing conditions. Each tank is divided into eight cubic sectionals (each 0.3 m^2), which holds three to four adult individuals. Sexes are continuously kept separate, since the presence of males can stress female behaviour. The only time males are placed with females is when a planned mating is scheduled. In this manner, we can document which particular male has mated with which female (and therefore, track fecundity of each female). Males are placed in the female cubical (usually at a 1:1 or 1:2 male to female ratio) and are removed anytime between 4 and 10 h. PVC pipe cut longitudinally is placed in the female cubical and used as artificial substrates for the females to lay their eggs after they have been mated.

Squids will mate and spawn in the tanks. Mating occurs without prior pair formation for *E. hyllebergi,* and controlled conditions (noting which pairs are mated, and how many times) are maintained for *E. tasmanica*. Spawning behavioural pattern is similar for both species. The male responds to the presence of a swimming female by initially approaching and then grasping her from below in a male to female neck position. The female is pulled down to the bottom where copulation takes place. Copulation takes 7–10 min and then the pair separate. Spawning is observed at dawn, 2–3 days after mating. Prior to spawning, the female investigates substrates for attaching her eggs by swimming around, and touching the substrata with the tip of her arm cone. In the tanks, the female attaches her eggs in clusters to the inner surface of the artificial substrates (Fig. 15.2). The time period for attaching is 40–60 s for one egg. Intervals between each egg attachment lengthens as the number of eggs increases, up to 2–3 min. Spawning is intermittent and irregular and may be extended over several weeks. The total number of eggs per female is about 100–470 with an average of 200 eggs. Females can spawn up to three to four clutches in her lifetime, with the number of eggs decreasing as the female becomes older (Steer et al. 2004; Nabhitabhata et al. 2005). For *E. tasmanica,* adults reared in captivity (F1 generation) live longer (2–3 months) and produce larger and more clutches per female. Wild-caught adult *E. tasmanica* at maturity produce approximately 3 clutches while in captivity, ranging from 25 to 100 eggs per clutch (with one exceptional female, which laid approximately 500 eggs in one clutch). F1

Fig. 15.2 Egg capsules of *Euprymna hyllebergi* attached to the inner side of the artificial substratum, a piece of cut polyvinyl chloride (*PVC*) pipe. (Photograph of J Nabhitabhata)

generation *E. tasmanica* females lay up to 5 clutches/lifetime, with sizes ranging from 100 to 250 eggs per clutch. Hatching rate from the F2 clutches is approximately 99% for the first clutch, with a decrease leading up to approximately 20% (~80% hatching rate) for later clutches. F1 adults are larger and thus far have lived for 1 year in captivity (Nishiguchi, unpublished). F2 adults have similar longevities, but hatching rates for the F3 generation was somewhat lower (70–80%)

15.4 Nursing of Eggs

15.4.1 Egg Characteristics

Eggs are single, stalkless and opaque white, having a droplet shape and calcareous leather-like coating capsule (Fig. 15.3). The size of each egg is about 4 mm along its major axis, 3 mm in its minor axis and weighs about 0.02 g. About 2 h after being laid, the outer coat (or capsule) turns brown, leathery and rigid in *E. hyllebergi* (Nabhitabhata et al. 2005), whereas in *E. tasmanica* the egg is orange from wild-caught specimens. In F1 and subsequent generations of *E. tasmanica,* egg capsules are white to opaque and remain so during development. This solid protection allows the developing embryo to become a "sessile organism" during the extended period of development (Boletzky 1998). Eggs are telolecithal. Asymmetric eight-cell cleavage occurs 10 h after fertilization. Clockwise rotation of the embryo occurs from days 3 to 8, at 28 °C. Organogenesis occurs from day 4. The unique bilobed character of the external yolk sac appears after day 5 when the capsule becomes more transparent and the embryo is now visible. Chromatophores develop from day 6, and four diverticula of the internal yolk sac from day 8. The first hatching occurs at day 12 (Fig. 15.4) for *E. hyllebergi,* and day 32 for *E. tasmanica*. The embryonic phase is about 12–18 days, after approximately 14 days at 28 °C for *E. hyllebergi,* and 21–28 days at 18 °C for *E. tasmanica*. The hatching period of eggs in the same clutch takes 5 days from first to the last eggs and primarily occurs on the third day. Average hatching rate is about 94% (82–100%) for both species.

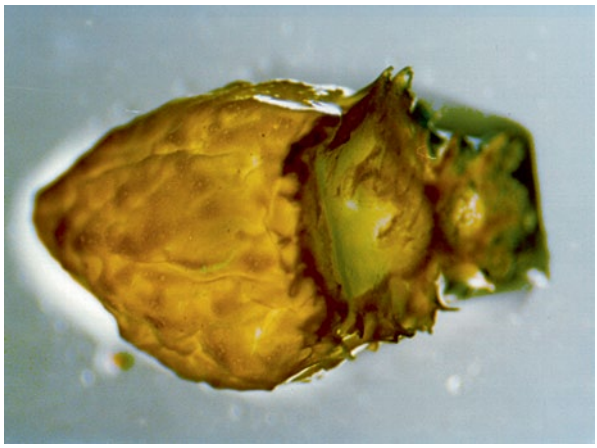

Fig. 15.3 Egg capsule of *Euprymna hyllebergi*; surface is colored brown by attached diatoms (40x). (Photograph of J Nabhitabhata)

Fig. 15.4 Hatchling of *Euprymna hyllebergi* (dorsum, 17x). (Photograph of J Nabhitabhata)

15.4.2 System Requirements and Management

Artificial substrates with egg clusters are transferred to hatch in fibreglass tanks of 50-L capacity containing 40-L filtered seawater. Two pieces of longitudinal-cut PVC pipe (25-mm diameter, 400-mm length) equipped with aeration devices are placed in each tank, facing in the same direction, to generate and direct an artificial current (Fig. 15.5). Tanks are cleaned by siphoning out the old water and replaced by a volume of 50 %. Temperature change is minimised by means of outside running water around the tank base (Nabhitabhata et al. 2005). The average temperature

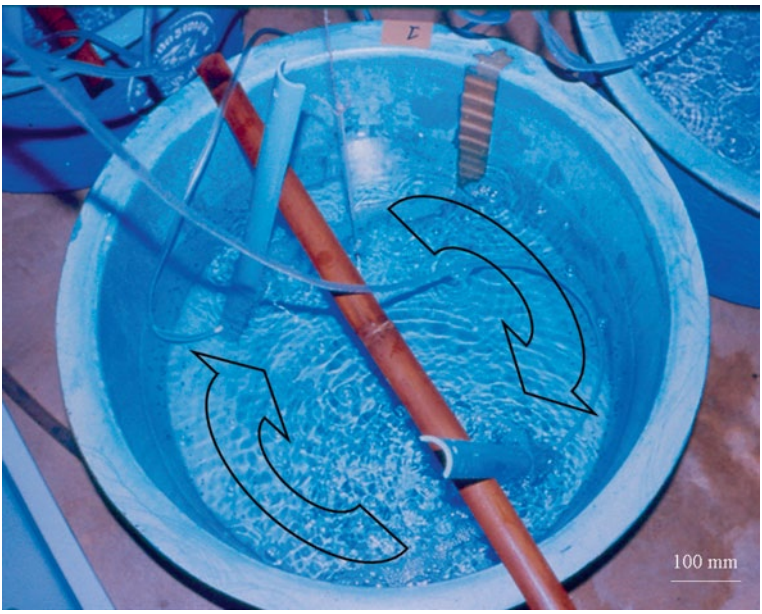

Fig. 15.5 A culture tank of *Euprymna hyllebergi* equipped with current generator devices (*arrows* indicate current direction). (Photograph of J Nabhitabhata)

can be maintained at approximately 28.2 °C, pH 8.0 and salinity at 32.5 psu. For *E. tasmanica,* clutches are placed in a 100-L polycarbonate tank with ultraviolet (UV)-filtered artificial seawater. Each individual clutch is placed in a cage made out of 2 pieces of PVC tubing, cut longitudinally and then glued back together with mesh netting in between. The clutches are placed in these cages so as not to have hatchlings mix with other clutches, as well as receiving enough aeration from below during development. Each cage is aerated with water from an individual spout that provides oxygenated seawater (Fig. 15.6). Water is kept at constant temperature (20 °C) with pH 8.0 and salinity 34.0 psu. Water changes are completed every other day to maintain salinity due to evaporation.

15.5 Nursing of Young

15.5.1 Hatchling Characteristics

The living mode of the hatchling includes a planktonic phase lasting from 6 to 8 h before the hatchling gradually adopts a benthic habit. The settling stage is approximately 5 days. Juvenile squids still enter the water column on a regular basis, alternatively planktonic and benthic, until 25–30 days after hatching. The internal yolk sac is still visible through the transparent mantle from hatching until the third day (Fig. 15.4).

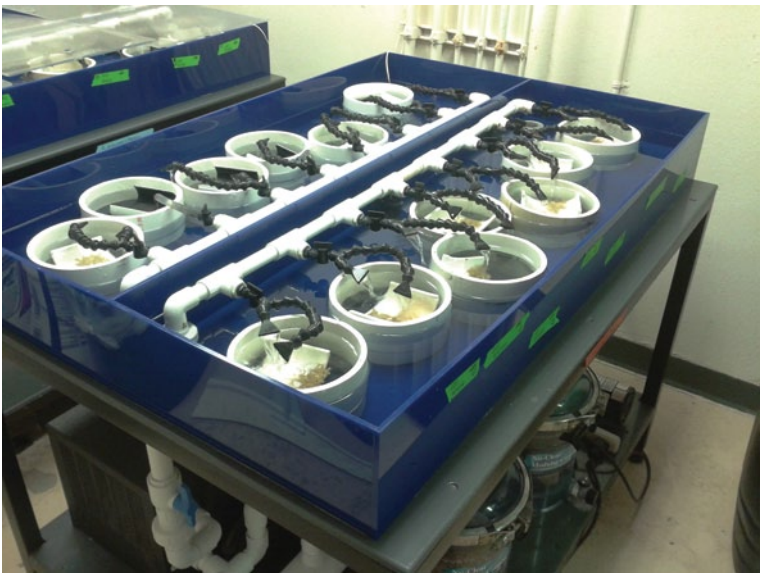

Fig. 15.6 Culture facilities for clutches/hatchlings for *Euprymna tasmanica*. (Photograph of MK Nishiguchi)

15.5.2 System Requirements and Management

Nursing of young is performed using the same system as for nursing of eggs for both species.

15.5.3 Feeding

The general task is to feed planktonic food to juvenile squids before the settling stage, at which time the squids are column feeders. Subsequently, benthic food is provided after the settling stage, when the juvenile squids settle to the bottom (Hanlon 1990; Hanlon et al. 1997; Nabhitabhata et al. 2005). *E. hyllebergi* hatchlings are fed with live, hatchery-produced penaeid shrimp larvae (*Penaeus merguiensis, P. monodon*) of the protozoea and mysis stages for 5 days after hatching (Fig. 15.7). Postlarvae of penaeid shrimps of the same species as well as wild mysids (*Mesopodopsis orientalis*) are also fed to the squids from hatching to 40 days. The planktonic young seize and eat its prey in the water column while hovering. After 25 days, the juvenile gradually changes to a benthic feeder, seizing its prey in the water column and then consuming it on the bottom substrate.

After 30 days, supplementary prey organisms for *E. hyllebergi* are palaemonid shrimps (*Palaemon styliferus*) and wild mysids (*Acetes* spp.). Training the squids to feed on dead fish meat (*Caranx leptolepis*) begins during this

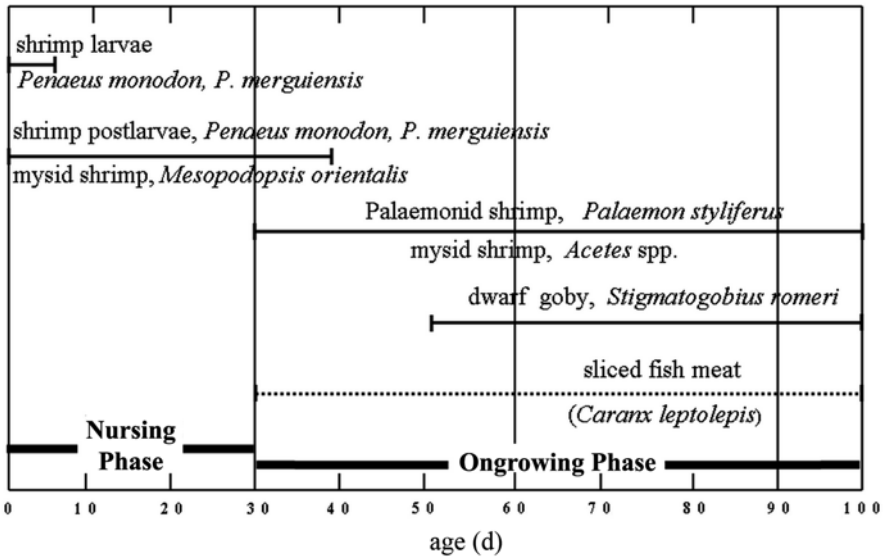

Fig. 15.7 Diagram of feeding of cultured *Euprymna hyllebergi* in the nursing phase (0–30 d) and ongrowing phase (after 30 d settlement) with live feed (*full line*) and dead feed (*dotted line*). (After Nabhitabhata et al. 2005)

period. Size grading is also initiated and continued every 10 days. The density is reduced from the initial 2–6 individuals L^{-1} by at least 25% after each grading (Nabhitabhata et al. 2005). *E. tasmanica* juveniles are fed on live, mysid shrimp for the first month (~30 days), i.e. *Tasmanomysis oculata, Paramesopodopsis refa,* and then are moved to smaller, post-larval panaeid shrimp (*Penaeus* sp.). Since *E. tasmanica* are larger when hatched, they are capable of obtaining bigger prey items earlier in their development than *E. hyllebergi*. Enriched brine shrimps (*Artemia parthenogenetica* and *A. franciscana*) can be used as substituted food when mysids are unavailable (Sinn 2005; Sinn and Moltschaniwskyj 2005; Sinn et al. 2008) although they are generally less preferred. *E. tasmanica* does not take dead prey, although there is no attempt to train juveniles to feed on this type of material.

15.5.4 Euprymna hyllebergi Growth

Hatchlings of Thai bobtail squid grow from about 2-mm ML and 0.004-g weight to 7-mm ML and 0.26 g in the first month (Fig. 15.8a, b; Nabhitabhata et al. 2005). The daily or instantaneous relative growth rate (IGR) is the highest between 10 and 20 days after hatching, about 5% in length and 17% in weight (Fig. 15.8b). The survival in the nursing phase from hatching to settling stage (0–30 days) is approximately 30%.

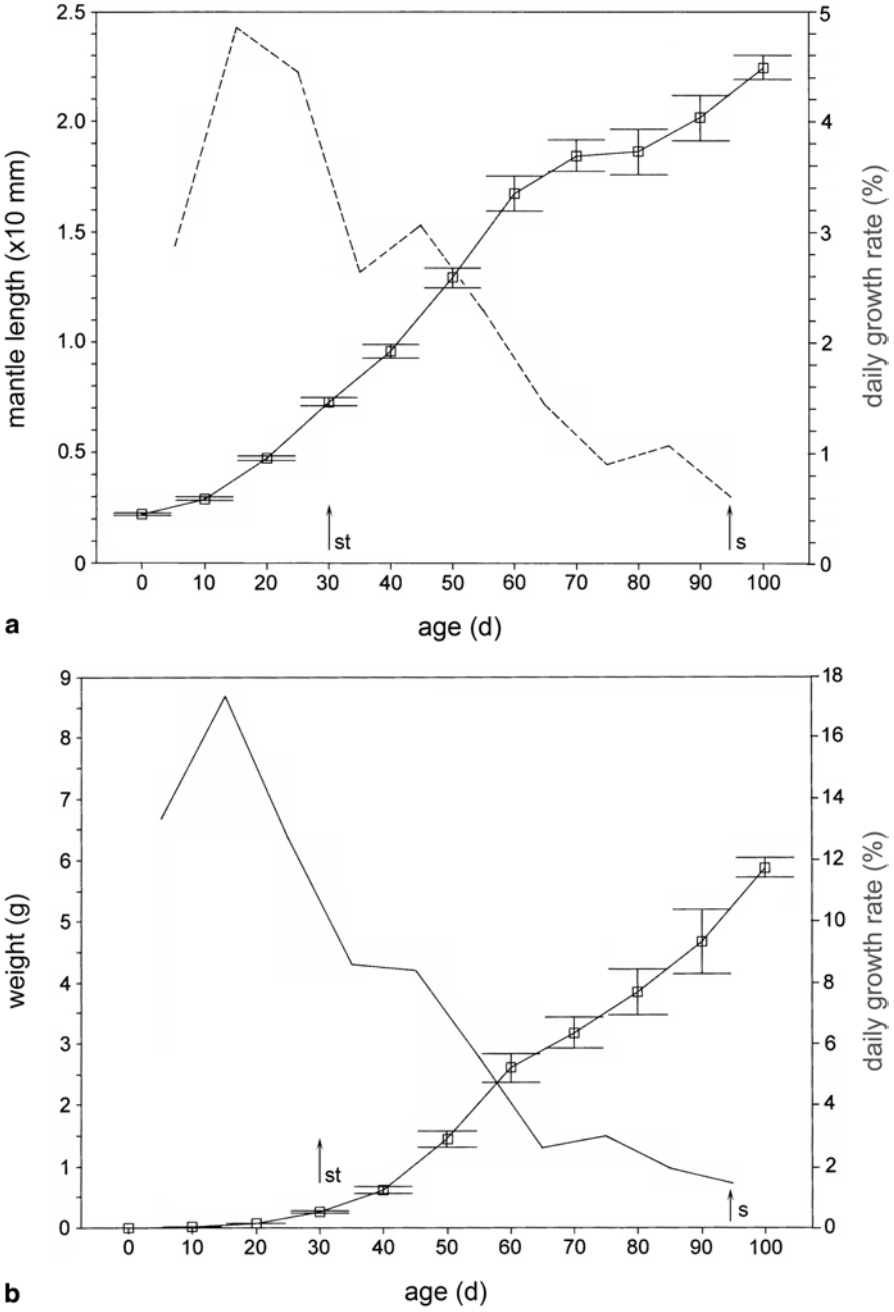

Fig. 15.8 Growth of *Euprymna hyllebergi* in terms of (*above*) mantle length (x10 mm), instantaneous relative growth rate (*IGR*: %) and age (*d*) after hatching and (*below*) weight (*g*), *IGR* (%) and age (*d*) after hatching. *Arrows* indicate spawning (*s*) and settling stage (*st*). (After Nabhitabhata et al. 2005)

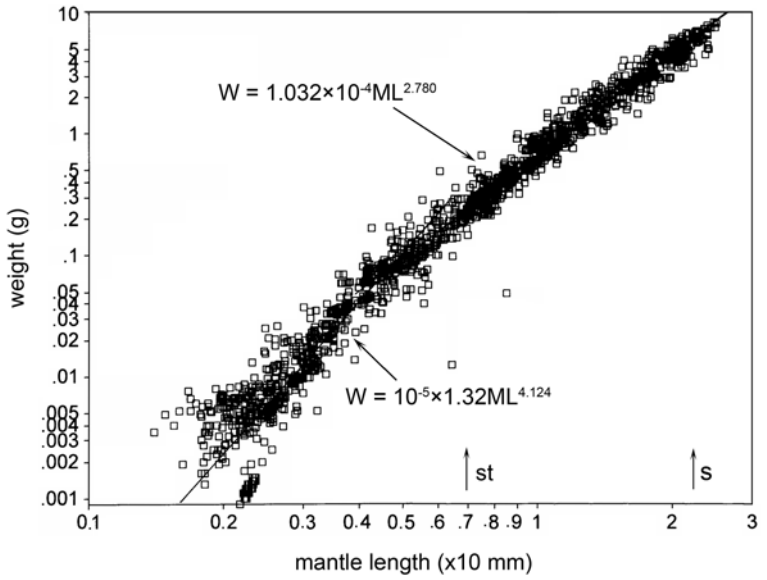

Fig. 15.9 Relationships between mantle length (x10 mm) and weight (*g*) of *Euprymna hyllebergi*; intercept of the two regressions at 5.5 mm mantle length. *Arrows* indicate spawning (*s*) and settling stage (*st*). (After Nabhitabhata et al. 2005)

Growth models demonstrate two phases of growth. The early phase was from hatching to 30 days where the relationships between the ML (mm) and weight (g) can be expressed as a power regression model (Fig. 15.9; Nabhitabhata et al. 2005):

$$W = 1.230 \times 10^{-4}\ ML^{4.124}. \tag{15.1}$$

The relationships between ML and age (d: days after hatching, Fig. 15.10; Nabhitabhata et al. 2005) and between weight (g) and age (d, Fig. 15.11; Nabhitabhata et al. 2005) can be expressed as the exponential models:

$$ML = 1.988e^{4.205\times10^{*}(-2)A} \tag{15.2}$$

$$W = 2.750 \times 10^{-3} e^{0.153A}. \tag{15.3}$$

15.6 Ongrowing

15.6.1 System Requirements and Management

For *E. hyllebergi,* ongrowing phase starts after the benthic young are able to accept dead fish meat. Tanks for ongrowing are the same tank used for the nursing phase and with similar management for both species. The density of *E. hyllebergi*

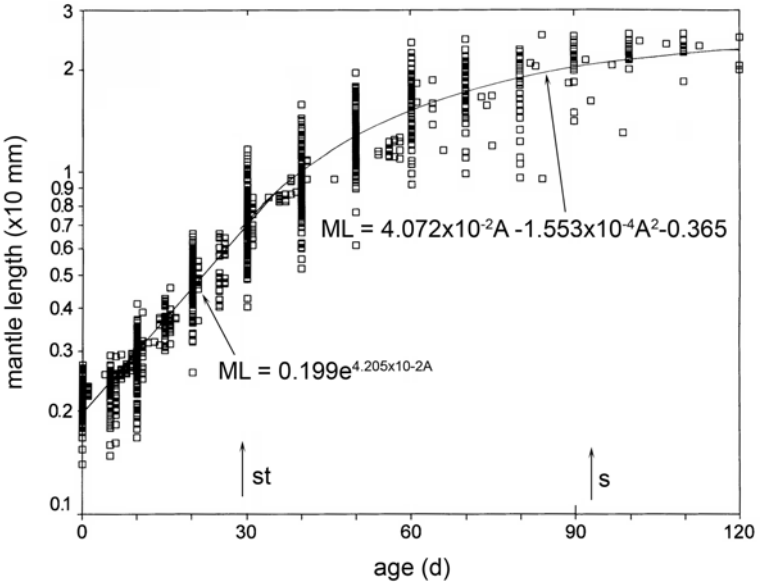

Fig. 15.10 Relationships between mantle length (x10 mm) and age (*d*) of *Euprymna hyllebergi*. *Arrows* indicate spawning (*s*) and settling stage (*st*). (After Nabhitabhata et al. 2005)

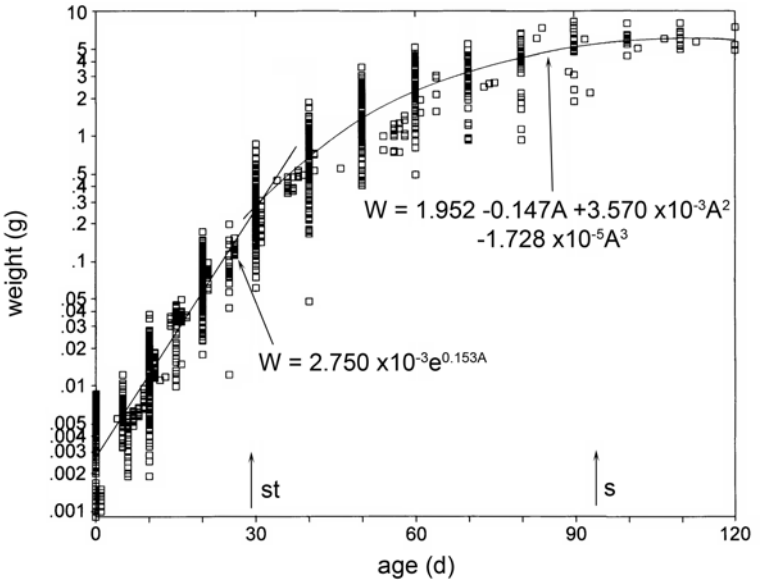

Fig. 15.11 Relationships between weight (*g*) and age (*d*) of *Euprymna hyllebergi*. *Arrows* indicate spawning (*s*) and settling stage (*st*). (After Nabhitabhata et al. 2005)

is changed from a water volume oriented to an (bottom) area oriented as 4–5 individuals m^{-2}. *E. tasmanica* juveniles are initially raised in round glass bowls (approximately 2 L) with sand at the bottom so the juveniles can settle. Unlike *E. hyllebergi* juveniles, *E. tasmanica* immediately settle on the bottom once they are hatched. Water is changed daily since the volume is small and there is greater evaporative loss from this volume. At approximately 2–3 weeks, juvenile squids are transferred to 40-L aquaria with sand on the bottom and raised until sexually mature (2 months). Generally, 20 squids are kept in an aquarium this size due to space limitations, but this number does not seem to affect their behaviour with any visible signs of stress. Since *E. tasmanica* F1 and F2 generations have higher growth rates than those caught in the wild, these individuals are moved earlier to the adult cubicals.

15.6.2 Euprymna hyllebergi *Growth*

The growth rate from hatching to 100 days of age for *E. hyllebergi* is approximately 2.4 % in length and 7.5 % in weight. At 60 days after hatching, the squid had grown to 17-mm length and 2.6-g weight and 22 mm and 6 g at 100 days. Food consumption of about 0.2 g d^{-1} or 37 % body weight d^{-1} enables calculation of the food conversion efficiency of about 37 % (range 14–99) from hatching to 100 days. This rate increases from 30 to 40 % after hatching to 60–70 % during 40–60 days with a peak of about 64 % between 50 and 60 days (Fig. 15.12). These values potentially relate to the storage of energy for the consequent reproductive period (Nabhitabhata et al. 2005). At 90 days after hatching, the survival from hatching is approximately 10 % and from settlement is 70 %.

Transition in growth phases is reflected in the nature of the growth models. The stage where the models shifted to a higher elevation is at about 30 days after hatching, and this corresponds to the observed settlement stage (Figs. 15.8–15.11). The second growth phase is from 30 to 122 days. The relationships between ML (mm) and weight (W, g) can also be expressed as a power regression model (Nabhitabhata et al. 2005) as happened in the early phase (Fig. 15.9):

$$W = 1.032 \times 10^{-3}\ ML^{2.780}. \tag{15.4}$$

The relationships between ML and age (d, days after hatching) and between weight (g) and age can be expressed as the quadratic equation (Fig. 15.10; Nabhitabhata et al. 2005) and a cubic regression model (Fig. 15.11; Nabhitabhata et al. 2005):

$$ML = 0.407A - 1.553 \times 10^{-3}\ A^2 - 3.648 \tag{15.5}$$

$$W = 1.952 - 0.147A + 3.570 \times 10^{-3} A^2 - 1.728 \times 10^{-5} A^3. \tag{15.6}$$

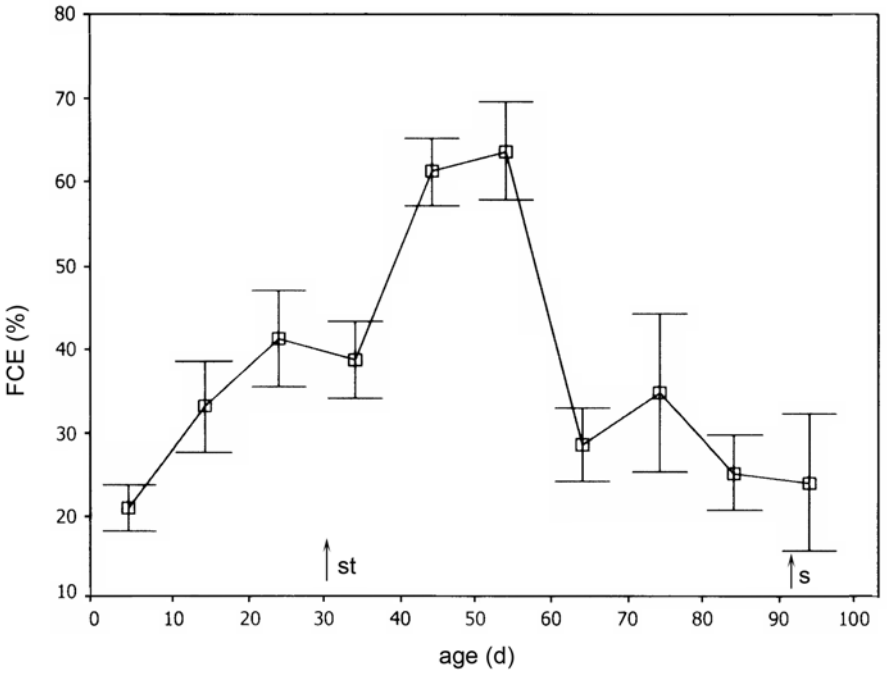

Fig. 15.12 Food conversion efficiency (*FCE*: %) of cultured *Euprymna hyllebergi* during growth (age: d). *Arrows* indicate spawning (*s*) and settling stage (*st*). (After Nabhitabhata et al. 2005)

15.6.3 Euprymna tasmanica *Growth*

Detailed studies on growth of *E. tasmanica* are scarce. Growth rate of *E. tasmanica* is rapid, with hatchlings reaching adult size in 2 months at 18 °C. *E. tasmanica* can grow from a ML of approximately 1.7 mm and a weight of 0.012 g at hatching (Steer et al. 2004) to 0.06 g at 21 days and to 6.8 g at 112 days (Sinn et al. 2008). The daily growth rate from day 21 to 63 is 7–9 %, from 63 to 84 days decreases to 2–4 % and from 84–112 days 1–2 % at 18 °C (Sinn 2005).

The relationships between weight and age are exponential from 7 to 44 days after hatching and linear from 58 to 140 days (Moltschaniwskyj and Carter 2010) which can be expressed as:

$$\mathrm{InW} = 0.069\mathrm{A} - 4.06 \tag{15.7}$$

$$\mathrm{W} = 0.07\mathrm{A} - 3.28. \tag{15.8}$$

15.7 Trends in Research and Industry

The main purpose of culture *Euprymna* is obviously not for human consumption. Present research in the fields of marine pharmacology, biotechnology and mimetic engineering requires small and “easy to culture” squids. Rapidly growing demand

in the ornamental aquaculture trade also requires organisms of similar characters, specifically size and ease of care. The small adult body size, benthic habit and good adaptability to culture conditions of *Euprymna* are prominent character suites that are well adapted to the aforementioned purposes. Based on such qualities, bobtail squids should be cultured on a small scale in order to reduce the cost of production. Additionally, a small-scale culture has advantages of the reduced size and benthic habits of the squids. The variety of flow-through open or closed seawater systems can yield different results and should be further studied to better quantify which systems are best for maximising production and those appropriate for each species.

Culture of *Euprymna* similarly encounters a bottleneck during the nursing phase similar to other cephalopods, since young innately feed on live feed. Future research should focus on developing feeds, both live and artificial. However, small-scale culture of live food organisms is more appropriate for small-scale culture of *Euprymna* in view of low operating costs at present. Development of artificial feed is necessary to reduce costs, but it could be postponed on a small scale. Artificial feed for cephalopods has not been commercially developed anywhere, but many studies are being completed, focusing on species that are aimed to be cultured as human food. Investigating various types of feed may give insight as to whether bobtail squids can also use artificial feed in such a manner.

E. hyllebergi and *E. tasmanica* can be cultured through multiple consecutive generations (3 generations for both *E. hyllebergi* and *E. tasmanica*) with similar growth rates (under similar conditions) without apparent effects of inbreeding on decreased growth (Nabhitabhata et al. 2005). Similar growth among generations enables a reliable supply of broodstocks for aquaculture and provides an alternative to continued fishing for wild-caught specimens, which can be time consuming and costly. However, the feasibility of inbreeding effects on decreasing of growth and fertility must be considered when producing future generations from the same broodstock. Broodstocks cannot rely solely on cultured batches, and wild broodstocks should be added intermittently to provide both genetic variation and possibly the induction of beneficial microbes that are necessary to keep squid healthy during their lifetime. Growth in captivity and culture methodology of both *E. hyllebergi* and *E. tasmanica* as well as their congeners should be further studied in views of maximising the aquaculture production and increasing our ability to provide a useful resource for a variety of research studies as well as the development of model aquaculture cephalopods.

15.8 Conclusions

The ability to maintain and grow small benthic squids such as *Euprymna* has opened up a new avenue for instigating the use of these animals as model systems in both bioengineering (adhesion) and biomedical (beneficial bacteria) research. The requirements for housing, maintaining and raising sepiolids is minimal and not as costly as other, more gregarious squid species, and this allows laboratories to set up facilities that may not necessarily be close to the ocean (such as NMSU). Presently, there are 14 laboratories in the USA alone that have culture facilities for raising

Euprymna; such facilities would not be feasible unless these animals were easy to transport long distances and maintained continually and without a nearby marine station or source of seawater. Additional research must be considered for the effects of inbreeding (when maintaining a constant broodstock) as well as comparing species for different traits that can be used for certain research foci. The inception of using cephalopods as research and not "feed" organisms is a new and exciting avenue that multiple areas of research can benefit from for furthering our knowledge in aquaculture, bioengineering, medicine, ecology and evolutionary biology.

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